Pronounced surface stratification of soil phosphorus, potassium and sulfur under pastures upstream of a eutrophic wetland and estuarine system

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Summary Text for the Table of Contents.

Movement of phosphorus off farms into waterways is detrimental to the health of downstream aquatic systems through promoting algal blooms. We measured the concentration of phosphorus, and other elements, in the top 100 mm of soil under a beef farm and a dairy farm and found extremely high concentrations in the top 10 mm of soil. Management practices that lower phosphorus concentrations in surface soil will likely benefit health of downstream ecosystems through reducing phosphorus movement off farm.

ABSTRACT

High concentrations of nutrients in surface soil present a risk of nutrient movement into waterways through surface water pathways and leaching. Phosphorus (P) is of particular concern, due to its role in aquatic system eutrophication. We measured nutrients under annual pastures on a beef farm and a dairy farm in the Peel-Harvey catchment, Western Australia. Soils were sampled in 10 mm increments to 100 mm depth in March, June and September. Plant litter contained ~300–550 mg kg^{-1} Colwell-extractable P. Extractable soil P was strongly stratified, being ~100–225 mg kg^{-1} (dairy) and ~50–110 mg kg^{-1} (beef) in the top 10 mm and < 40 mg kg^{-1} at 40–50 mm depth. Total P and extractable potassium were also highly stratified, while sulfur was less strongly stratified. Shoot nutrient concentrations indicated that nitrogen was often limiting and sulfur sometimes limiting pasture growth: concentrations of P were often much greater than required for adequate growth (>4 mg g^{-1}). We conclude that high P concentrations at the soil surface and in litter and shoots are a source of risk for movement of P from farms into waterways in the Peel-Harvey catchment.

Keywords: nutrient stratification, pH, organic matter, eutrophication, surface soil

INTRODUCTION

Eutrophication of naturally phosphorus (P)-limited waterways and standing waters due to the movement of P off farmland is a significant environmental problem on a global scale (Sharpley *et al.* 2015) and is not easily remediated (Jarvie *et al.* 2013; Rivers *et al.* 2013). Farmland may contribute P from diffuse sources, such as large areas of land with high concentrations of readily-mobile soil P due to past fertiliser applications, and from point sources, such as the slurry from intensive livestock enterprises (Weaver and Read, 1998; Rivers *et al*. 2013). Diffuse P sources present the greatest problem, as they are the most challenging to understand and amend. Worldwide, extensive research has been undertaken with the aim of reducing the contribution of diffuse P sources to eutrophication (Sharpley *et al*. 2015). However, the application of this research has proved problematic, and one of the many reasons is an incomplete understanding of the specific pathways for P movement within the farm and off the farm into waterways in the target environment (Sharpley *et al.* 2015).

In the coastal plain of the Peel-Harvey catchment in south-western Australia, forests and woodlands have been largely replaced with pastures consisting of winter-active annual species, which support beef and dairy enterprises (Ruprecht and George 1993). The area has a long-term mean annual rainfall of around 1000 mm, which is strongly winter-dominant (i.e. ~54% falls in winter and ~ 4% in summer). The area also mostly has a low surface gradient and, therefore, an extensive network of shallow open drains has been constructed to mitigate waterlogging and inundation (Cooper 1979). These open drains efficiently move surface water from upstream farms into shallow estuarine waters, saline, brackish and freshwater wetlands, and several lake systems including a ‘Wetland of International Importance’ under the Ramsar Convention on Wetlands (Hale and Butcher

2007). The movement of P into these ecosystems has long been recognised as a major contributor to eutrophication and algal blooms (Lukatelich and McComb 1986), which undesirably affect amenity, water quality and wildlife. Moreover, the Peel-Harvey catchment is part of a terrestrial biodiversity hotspot and many of its native plant species are adapted to low soil P and are poorly competitive if soil P increases (Lambers *et al*. 2013). The movement of P through overland flow or shallow groundwater flow from farms into remnant native vegetation is, therefore, also a matter of concern.

The Environmental Protection Authority of Western Australia has set a goal of reducing the movement of P into the Peel-Harvey estuary by ~50% (EPA 2008), but recent modelling suggests that P inputs from the watershed will continue to increase (Rivers *et al.* 2013). An incomplete understanding of how P moves from farms into waterways has impeded the design of management strategies to reduce P losses from soils in this catchment. Point sources of P, such as piggeries, are easily identified and relatively well understood. Diffuse sources are not as easily mapped or well understood, but are believed to contribute the most P into the estuary (Rivers *et al.* 2013). The major diffuse source of P is pastures (beef and dairy). These have been fertilised for many years, primarily with inorganic P fertiliser, and are consequently greatly enriched with P (Rivers *et al.* 2013) due to accumulation in the surface soil of P in excess to pasture requirements in both inorganic and organic forms (McLaren *et al*. 2015). The large area of pastures in the catchment accounts for most of the 2000 tonnes of P that are applied each year. Although losses of P per hectare are small they are estimated to contribute around 140 tonnes of P per year to the estuary (Rivers *et al*. 2013). Modifying farm management to reduce the movement of diffuse-source P into the open drains requires a detailed understanding of the distribution of P on these farms and the means by which P moves off-farm. Other nutrients have also

been supplied to pastures, notably potassium (K) and sulfur (S). Lesser enrichment with K and S has likely resulted in limitations of K and S to pasture growth and P use (Weaver and Reed 1998).

In the Peel-Harvey catchment, leaching of P into shallow water tables is often the primary path of P movement into waterways and open drains located at low points in the landscape (Weaver and Summers 2014). However, in areas with very limited slope and/or subsurface impediments to groundwater flow, the movement of P through surface water pathways (infiltration excess or saturation excess overland flow and, perhaps, return flow of infiltrated water) may dominate, particularly once the soil profile is saturated (Ruprecht and George 1993). In pastures, high concentrations of P on the soil surface due to top-dressing of fertiliser, manure and infrequent tillage would increase the likelihood of P movement into the open drains (Dougherty *et al.* 2006). Indeed, several studies in other regions have reported high concentrations of P in the soil surface layers of pastures (0–40 or 0–50 mm) (McLaughlin *et al.* 1990; Haynes and Williams 1992; Cayley *et al.* 2002; Dougherty *et al.* 2006) which suggests that management strategies to reduce the movement of P should focus on this part of the profile. However, it is not well known whether these concentrations change over time as could occur due to management such as heavy periods of intensive grazing or fertiliser addition, or seasonal change such as growth of annual pastures or heavy winter rainfall. Furthermore, the construction of soil P risk frameworks for this catchment (e.g., soil P change point – Heckrath *et al.* 1995) would likely be complicated by a heterogeneous distribution of nutrients and buffering materials through the soil profile.

In view of the above, the aim of this study was to examine the stratification of P within the top 100 mm of soil in three permanent pastures in the Peel-Harvey catchment to identify whether P is highly stratified and present in high concentrations at the top of the soil profile. We also examined whether this stratification differs for pastures under contrasting management or on differing soil types and whether it changes during the course of the winter growing season. Three pastures were sampled: two mixed-composition annual pastures on a relatively low-intensity commercial beef farm (one on a deep sand and the other on a sandy loam over clay) and one annual pasture of ryegrass (*Lolium rigidum* L.) on a sand over clay on a high-intensity dairy farm (Fig. 1). The study builds upon previous studies of nutrient stratification in pastures and crops (McLaughlin *et al.* 1990; Haynes and Williams 1992; Cayley *et al.* 2002; Dougherty *et al.* 2006; Vu *et al*. 2009; Saarela and Vuorinen 2010; Haygarth *et al*. 1998) by including, in a single study, three sampling times within the growing season, small (10 mm) soil depth increments and measurements of extractable potassium and sulfur, pH and soil organic matter in addition to extractable and total P. The paddocks were under commercial management during the study.

MATERIALS AND METHODS

Environment

Pastures were sampled on two commercial farms located approximately 8 km apart (Fig. 1A), close to the town of Waroona in the coastal plain of the Peel-Harvey catchment in Western Australia. Both farms were likely established around 100 years ago following clearing of natural vegetation and soils are infrequently tilled. Fertiliser inputs across the region increased substantially after world war two (Birch, 1982; Cooper, 1979). The Peel-Harvey catchment has a typical Mediterranean climate with hot, dry summers and mild, wet winters (Table 1). Non-irrigated pastures such as the ones sampled for this study are,

150 therefore, largely based on winter-active annual pasture species. The two farms were
151 chosen to be representative of two common, but contrasting, farming enterprises in the
152 coastal plain: low-management-intensity beef enterprises and high-management-intensity
153 dairy enterprises. Across the catchment, these two enterprise types contribute greater than
154 75% to fertiliser P inputs and to P entering the estuary (Rivers *et al*. 2013).
155
156 *Rainfall*
157 The monthly rainfall totals were obtained from the nearby town of Waroona (32.85°S,
158 115.92°E, 40 m elevation, Bureau of Meteorology station 009538) (Table 1). In the year
159 before the study, 2011, total rainfall was similar to the long-term average. In 2012,
160 monthly rainfall totals were close to long-term averages, except for July which received
161 only 47 mm compared with the long-term average of 188 mm.
162
163 *Beef cattle farm (two sample sites)*
164 The beef farm had previously been a mixed-beef and dairy enterprise, but had soley been a
165 beef-beef enterprise for more than 20 years at the time of sampling. The pasture we
166 sampled was located on a small 3–4 m high dune of sand overlying clay, a common
167 landform in the area. The farm was managed in a relatively low-intensity manner with an
168 annual addition by top-dressing onto growing pastures of 100–200 kg ha^{-1} of mineral
169 fertiliser (7% P, 2% K, 2% S, 26% Ca, 1% Mg) and infrequent application of locally-
170 sourced lime-sand at ~1 t ha^{-1}. No nitrogen (N) fertiliser was applied as the farmer relied
171 upon annual legumes, primarily *Trifolium subterraneum* L., to biological fix N. The
172 pasture contained a mix of weeds, pasture legumes and grasses, including some summer-
173 active perennials, which were grazed by set-stocked cattle. Two sites, around 25 m apart,
174 were sampled (Fig. 1B). The ‘upslope’ site was at the top of the dune where the soil is a

175 Gavin sand (McArthur and Bettenay 1959). The major pasture species present during
176 winter were *Arctotheca calendula* (L.) Levyns, *Lolium rigidum* L., *Lupinus cosentinii*
177 Guss., *Cynodon dactylon* (L.) Pers. and *Bromus* spp. The 'midslope' site was lower on the
178 dune, close to the break of the slope towards a flat area. The soil is a Mayfield series sandy
179 loam over clay (McArthur and Bettenay 1959) and the major pasture species present were
180 *A. calendula*, *L. rigidum*, *Lotus subbiflorus* Lag., *T. subterraneum* and *Pennisetum*
181 *clandestinum* Chiov. At the bottom of the dune on which the two sites were located is the
182 Mayfield drain (Fig. 1B), an open drain approximately 3 m wide and 1.6 m deep. Shallow
183 groundwater enters this drain via seepage from the bank and, more significantly, surface
184 water enters the drain by overland flow.
185
186 *Dairy cattle farm (one sample site)*
187 The dairy farm had been under continuous management as a dairy for ~30 years. The
188 pasture we sampled was located on a Coolup series sand over clay (~0.4 m) (Fig. 1C). It
189 was re-sown annually with *L. rigidum* using zero-tillage techniques and contained a small
190 amount of *Poa annua* L. and *T. michelianum* Savi. A dairy herd rotationally grazed the
191 pasture on a tightly-controlled cycle whereby paddocks were temporarily divided to allow
192 stock access to adequate pasture for a single day; this equated to three or four days grazing
193 in a paddock every month. In-season N management was a priority with ~40 kg ha^{-1} of N
194 as urea top-dressed onto pastures after each grazing event. Lime (2.5 t ha^{-1}) was applied
195 before the first heavy rains of the 2012 growing season on paddocks with acid soils to
196 maintain the soil pH at 5.5. Extractable soil P concentrations were high due to past
197 application of P fertiliser and, consequently, the farmer had not applied P fertiliser for
198 around five years. However the farmer did apply an inorganic fertiliser containing P in
199 2012 (details unknown). Groundwater and, in particular, surface water from this site drain

200 into the South Coolup Drain (Fig. 1C), an open drain approximately 5 m wide and 1.7 m
201 deep (Steele, 2008). Overland flow discharges via shallow-interconnected surface
202 depressions (see arrows in Fig. 1C). More detail on drainage characteristics and nutrient
203 export from the South Coolup Drain can be found in Steele (2008) and EPA (2008).
204
205 *Sample collection*
206 Samples were collected in March, June and September 2012. The March sampling
207 preceded the first heavy rains of the growing season and there was little or no green pasture
208 present. Three quadrats (0.5 × 0.5 m) were randomly placed within each of the three sites
209 at each time of sampling; areas that had obviously recently received urine or dung were
210 avoided. Shoots were cut close to the soil level and removed. Green shoot material in
211 contact with the soil and senesced shoot material at the soil surface were removed
212 (hereafter referred to as 'litter'). A custom-made 'plane' was then used to remove the soil
213 in 10 mm increments down to 100 mm depth and samples were transported to the
214 laboratory on the day of sampling.
215
216 *Sample analyses*
217 Soil samples were oven-dried at 40°C for one week and sieved to 2 mm. A subset of
218 increments was analysed (i.e. 0–10, 10–20, 20–30, 30–40, 40–50, 70–80, 90–100 mm) by
219 CSBP laboratories (Bibra Lake, Western Australia). Unless otherwise specified, the
220 methods for soil analysis followed those of Rayment and Lyons (2011) and codes from this
221 reference are supplied: total P (9A3b); bicarbonate-extractable P (9B) and potassium (K)
222 (18A1, Colwell (1965)); P-buffering index (PBI) (912C, Allen and Jeffrey (1990));
223 extractable sulfur (S) (10D1; Blair *et al.* (1991)); pH in a soil:solution ratio of 1:5 (4A1,
224 4B3, 3A1); and organic carbon (6A1, Walkley and Black (1934)).

225

226 The litter samples were treated as soil samples for analysis as they were a mixture of plant
227 material and surface soil. Samples were dried at 60°C to a constant weight. For extractable
228 P, samples were ground to <2 mm and 1 g shaken with 100 mL of 0.5 M $NaHCO_3$ (pH 8.5)
229 for 16 hours (Colwell 1963), centrifuged, and the P in the clear supernatant measured
230 colorimetrically (Murphy and Riley 1962). For total P, samples were ground to <0.5 mm
231 and 0.3 g digested in nitric/perchloric acid (Kuo 1996), and the P measured
232 colorimetrically (Murphy and Riley 1962).

233

234 The pasture shoots (June and September only) and litter (March and September only) were
235 dried at 70°C to a constant weight and then ground. Shoot samples of approximately 100
236 mg were digested in nitric/perchloric acid and analysed for P, K and S using inductively-
237 coupled plasma atomic absorption with a Perkin Elmer Optima 5300 DV optical emission
238 spectrometer (OES; Shelton, CT, USA). Nitrogen concentration was determined by dry
239 combustion using an elemental CN analyser (Elementar Analysensysteme GmbH, Hanau,
240 Germany).

241

242 *Data analyses*

243 Soil plane data were analysed with three-way ANOVA using Genstat version 14.1 (Lawes
244 Agricultural Trust, Rothamsted Experimental Station, Harpenden, UK). The factors
245 examined were site (beef midslope, beef upslope, dairy farm), depth (0–10, 10–20, 20–30,
246 30–40, 40–50, 70–80, 90–100 mm) and time (March, June, September) and their
247 interactions. Most data required log10 transformation to meet the assumption of normality.
248 No outliers were removed except for the extractable P, total P and extractable K data from
249 one quadrat at the dairy farm in September 2012 because very high concentrations at all

depth increments were interpreted as likely resulting from a feeding station previously being present (215-464 mg kg^{-1} extractable P, 677-1333 mg kg^{-1} total P, 152-679 mg kg^{-1} extractable K). The litter and shoot nutrient concentration data were analysed with two-way ANOVA. The two factors examined were site (beef midslope, beef upslope, dairy farm) and time (March and September for the litter; June and September for shoots) and their interactions.

RESULTS

The results from the soil planes are presented in Figures 2–4. All variables were strongly affected by depth ($P<0.001$), with the exception of PBI (Table 2). Sampling time strongly affected pH and extractable K ($P<0.001$) as well as total P, S, and organic carbon ($P=0.01$-0.018). Site affected all variables ($P\leq0.004$). There were numerous interactions among factors.

Extractable P concentration was highest at the dairy site ($P<0.001$) and declined sharply with soil depth ($P<0.001$); indeed by 40–50 mm depth, the concentration of extractable P was generally half or less than that at 0–10 mm depth (Fig. 2A, Table 2). There was an interaction between soil depth and site ($P=0.043$), as the decline with depth was most marked for the dairy site where the extractable P concentration at the three sampling times was ~100–230 mg kg^{-1} at 0–10 mm, but only ~40 mg kg^{-1} by 30–40 mm depth. There was also an interaction between site and time ($P=0.038$) driven largely by the dairy site where extractable P concentration was lowest in June and highest in September.

The concentration of total P also declined greatly with depth ($P<0.001$) and was affected by sampling time, being lower in March than in June and September ($P=0.018$) (Fig. 2B,

Table 2). The concentration of total P was higher at the dairy and beef midslope sites than at the beef upslope site ($P<0.001$). At the beef upslope and dairy sites, total P was more than 200 mg kg^{-1} higher at the top of the profile in June than in March. The effect of sampling time on extractable P concentration did not always mirror total P concentration; for instance, the very high extractable P concentration in the top 10 mm of the profile in September at the dairy farm was not reflected in the total P concentration.

PBI was measured only in June and September, and was higher at the beef midslope site than at the beef upslope or dairy sites ($P=0.004$) (Fig. 2C, Table 2). PBI was unaffected by depth or sampling time ($P>0.05$). PBI was generally low (i.e. 20–50), although some samples from the beef farm were moderate (i.e. 50–100). There was large variation among the three replicate quadrats for the beef upslope and beef midslope sites, hence the three quadrats from each site are presented individually. The variation among the replicate quadrats at each site resulted from one quadrat having higher or lower PBI at all depths. The beef farm quadrats with higher PBI (i.e. >40) also had a higher total P concentration, and thus there were large standard errors for mean total P (Fig. 2B). For the beef farm, where PBI was variable, there was a significant linear positive correlation between PBI and total P concentration for the upslope and midslope sites (both $r^2=0.66$, $P<0.001$) and a significant correlation between PBI and extractable P concentration for the upslope site only ($r^2=0.32$, $P<0.001$).

The dry mass of litter on the soil surface was affected by an interaction of site and time ($P=0.006$), being similar at all three sites in March, but greatly reduced by September at the beef farm, but not the dairy (Table 3). The extractable P concentration for the litter ranged from 309–559 mg kg^{-1} and was not affected by site or sampling time ($P>0.05$). The total P

concentration of the litter ranged from 525–826 mg kg^{-1} and differed among sites (P<0.001) being lowest for the beef midslope site and highest for the dairy site. There was no relationship between total P concentration in the litter and total P concentration in the top 10 mm of soil (P>0.05). However, there was a positive linear relationship between total P concentration in the litter and extractable P concentration in the top 10 mm of soil (R^2=0.45, P=0.005). On an area basis, the extractable and total P contained in the litter were both always less than 1.5 kg ha^{-1} and highest in March (P≤0.02).

Soil sulfur concentration decreased strongly with depth (P<0.001) and was highest at the dairy site (P<0.001) (Fig. 3A; Table 2). Sulfur concentration also decreased over the sampling season (P=0.01), particularly at the dairy site (P=0.015). Soil extractable K concentration also decreased strongly with depth (P<0.001) and decreased over the sampling season (P<0.001), with the decrease from June to September greatest for the beef upslope site (P=0.021). In general, soil extractable K concentration was lowest at the dairy (P<0.001) (Fig. 3B). The beef farm quadrats with a high PBI also had high S and extractable K concentrations and this resulted in large standard errors at some sampling times (e.g. beef upslope for June).

Soil pH was affected by a strong three-way interaction of depth, sampling time and site (P=0.005) (Fig. 4A, Table 2). Soil pH declined with sampling depth at all sites, being around one pH unit lower at 90–100 mm than at 0–10 mm. There were also strong effects of sampling time of ~0.5 to 1 pH unit, but these differed among sites and soil depths. For instance, pH was highest in September at the beef farm sites, but highest in June at the dairy and these seasonal changes occurred at all soil depths (0-100 mm) at the beef upslope and diary sites, but not the beef midslope site. In general, soil pH was lowest at the dairy.

Soil organic carbon decreased with depth ($P<0.001$), decreased over time ($P<0.001$) and was lowest at the beef upslope site, intermediate at the beef midslope site and highest at the dairy ($P<0.001$) (Fig. 4B, Table 2). An interaction among site and sampling time ($P=0.03$) resulted from the greatest increase occurring from March to June for the beef upslope and dairy sites, but from June to September for the beef midslope. The large standard errors at the beef farm resulted from quadrats with higher organic carbon at all depths and, again, these quadrats were those with a high PBI.

Pasture shoot nutrient concentrations were measured on a bulked sample that consisted of all species present in the quadrat (Table 3). Shoot P concentration was lower for the beef midslope site than for the other two sites ($P<0.001$) and lower in September than in June ($P=0.002$). Shoot K concentration showed a similar trend, but this was not significant ($P>0.05$). For shoot S concentration there was an interaction between site and sampling time ($P=0.013$) as it did not differ among sites in June, but in September the shoot S concentrations at the beef farm sites were lower than the dairy values. Shoot N concentration was measured only in September and differed with site ($P=0.004$) being very low for the beef farm sites (11–13 mg g^{-1}) compared with the dairy farm (27 mg g^{-1}).

DISCUSSION

Did P, K and S concentrations and other soil properties vary among sampling times?

For the soil nutrients we examined, the effects of sampling time were mostly small (variance ratio 2-11) compared with the effects of depth (variance ratio 14-46). Most large differences among sampling times occurred in the top 10 mm of the soil profile. Some effects of sampling time are perhaps related to the addition of fertiliser during the growing

season, likely a single application on each farm, or sampling following an intensive period of grazing (e.g., the higher extractable P and S concentrations in the surface 10 mm at the dairy farm in September). However, for extractable P, the explanation may be more complex. At the dairy farm in September, the increase in extractable P concentration of ~100 mg kg^{-1} compared with June was far greater than could be expected from even a generous rate of fertiliser application, and was not reflected in an increase in total P. Several factors may have contributed to this increase including P mineralisation processes, perhaps associated with soil being waterlogged at the time of sampling (Bradley *et al*. 1994) and warming spring temperatures. In addition, significant mobilisation of P likely occurred during rainfall events due to leaching from the large biomass of pasture shoots that accumulated before each short grazing period. Most of the P in such green plant material is water soluble and, if leached, available to plants and soil microbes (Bromfield and Jones 1972; Noack *et al.* 2012), where some organic forms will be readily mineralised (Nash *et al.* 2014). Support for this contention comes from McDowell *et al.* (2007) who, using simulated rainfall, found that runoff from plots with membranes covering the soil (i.e. where only growing plants could contribute to nutrient load) contributed around half of the dissolved reactive P of plots where the soil interacted with the rainfall. Finally, seasonal variation in soil P pools may also result from turnover in soil microbial biomass in response to processes such as waterlogging (dairy farm) and wet/dry cycles, especially at the end of a hot, dry summer period (dairy and beef farms) (Sparling *et al*. 1985; Blackwell *et al*. 2009).

In our study, soil organic matter content tended to increase as the season progressed; this was particularly obvious for the dairy farm at 50–100 mm depth. Presumably this trend reflects the proliferation of roots of the mainly annual pasture species as the growing

season progressed as well as senescing plant material accumulating at the soil surface. Soil pH also increased by 0.5–1 units at the beef farm between March and September which was most evident in the top 0–50 mm. This increase is consistent with the return of organic matter alleviating acidity due to the growth of annual pastures and the return of senesced/trampled plant material to the soil surface.

Were P, K, S concentrations and other soil properties stratified?

At the beef and dairy farms, extractable P and total P were all highly stratified, resulting in high concentrations at the soil surface. For extractable P, similar stratification was reported for *T. subterraneum*-based pastures in south-eastern Australia (McLaughlin *et al.* 1990), pastures consisting of a mix of *Lolium perenne* L. and *T. repens* L. in New Zealand (Haynes and Williams 1992), fertilised perennial grass pastures in Finland (Saarela and Vuorinen 2010), and grass swards dominated by *L. perenne* in southwest England (Haygarth *et al*. 1998). In our study, the greatest stratification was for total P at the dairy farm where concentrations ranged from ~600–800 mg kg^{-1} at 0–10 mm to <200 mg kg^{-1} at 90–100 mm depth. The dairy farm had the highest concentrations of total and extractable P, reflecting a history of higher fertiliser inputs than the beef farm due to around 30 years of continuous operation as a dairy. Potassium was also highly stratified. However, S was stratified to a lesser extent, which agrees with Coad *et al.* (2010) and may reflect its greater mobility in soil (Watson 1969).

The pH of the soil decreased with depth in the top 100 mm of soil by close to one pH unit. Such pH gradients develop quite quickly under field conditions, within 5–7 years of surface soil being mixed (Conyers and Scott 1989; McLaughlin *et al.* 1990). Indeed, Evans *et al*. (1998) found that stratification developed during a single growing season, due to a

400 decrease in pH below 20 mm, and attributed this to nitrification followed by nitrate
401 leaching. Organic carbon concentrations also markedly decreased with soil depth. This
402 change was similar to that reported under crops and pastures in south-eastern Australia
403 (McLaughlin *et al*. 1990; Vu *et al*. 2009), and is presumably largely a reflection of return
404 of plant material to the surface of the soil.
405
406
407 *What caused the stratification of nutrients?*
408 The positive correlation between PBI and total P concentration at the beef farm resulted
409 from some individual profiles having a higher PBI throughout the profile and hence
410 enabling greater amounts of extractable P to be tightly bound. However, the very high
411 extractable P concentrations in the soil surface layers indicates that this binding capacity
412 had been saturated and that, consequently, P had accumulated as extractable P. Overall, the
413 high concentrations of P and other nutrients at the soil surface likely result from the
414 continuous input to the soil surface of plant material and animal dung (for P) and urine (for
415 K) (Haynes and Williams 1992), as well as fertiliser (Haynes and Williams 1992; Saarela
416 and Vuorinen 2010; McLaren *et al.* 2015, 2016). Maintenance/increase of these high
417 surface concentrations is also exacerbated by continuous cycling of P between
418 aboveground and belowground pools as illustrated by the strong positive linear relationship
419 between total P concentration in the litter and extractable P concentration in the top 10 mm
420 of soil.
421
422 *Is stratification a problem?*
423 The high concentrations of P at the soil surface undoubtedly present a significant risk for P
424 loss during the winter wet season (Haygarth *et al*. 1998; Dougherty *et al.* 2006; Melland *et*

425 *al.* 2008) by surface water pathways (Simmonds *et al.* 2016), and also preferential flow
426 pathways in the soil profile or leaching through the soil matrix (likely at the beef farm
427 upslope); the latter may become prominent in the winter growing season, when surface soil
428 becomes saturated and evapotranspiration is low. The very high concentrations of
429 extractable P in the top 10 mm of the soil profile (i.e. up to ~225 mg kg^{-1}) may exacerbate
430 P movement if they are above the "change point" (Heckrath *et al*. 1995). The change point
431 has not been determined for these soils. However, key challenges for using it, or other soil
432 character-defined parameters, to define the risk of P movement off pastures in the Peel
433 Harvey arise from the high P stratification in the top 100 mm of the soils and the variation
434 in PBI over small scales. As mixing contrasting samples from depth increments or
435 replicate profiles likely substantially changes results, we suggest that further work is
436 required to adapt risk prediction for stratified, variable soils.
437
438 *How can stratification be reduced?*
439 Several management practices have been proposed as a means to reduce P loss from farms
440 into waterways in the Peel-Harvey catchment. These include using perennial pastures,
441 managing waterways and riparian vegetation, only applying P fertiliser if soil tests indicate
442 P is limiting for pasture growth, using less-soluble fertilisers, applying fertiliser onto
443 growing pastures and using P-retentive soil amendments (Rivers *et al.* 2013). Our finding
444 of a high surface concentration of nutrients suggests that other management practice
445 changes should be considered.
446
447 Selection of more P-efficient pasture cultivars is one possible change. Species and cultivars
448 may differ greatly in shoot P concentrations at a given level of extractable soil P, and in
449 external critical P requirements, with grasses generally presenting lower shoot P

450 concentrations and a lower external critical P requirement than legumes (Ozanne *et al.*
451 1969; McDowell *et al.* 2011; Sandral *et al.* 2015; Haling *et al.* 2016). Thus, decreasing the
452 rate of P-fertiliser addition to pastures to match the P requirements of grasses only, or of
453 annual legumes with lower P requirements than the current widely-used species *T.*
454 *subterraneum*, would reduce the size of the pool of readily-soluble P circulating through
455 plants and top soil layers and thus reduce the risk of losses. As applying P fertiliser above
456 the rates required for pasture maintenance causes accumulation of sparingly-soluble forms
457 of organic and inorganic P in the soil (McLaren *et al.* 2015), reduced rates of P fertiliser
458 application would also improve the P use efficiency of the farming systems.
459
460 Mixing of soil through tillage or soil inversion may reduce the risk of nutrient loss from
461 top soil layers through surficial processes by reducing nutrient concentrations at the soil
462 surface (Vu *et al*. 2009) and in the soil water at the surface (Nash *et al.* 2015). Soil
463 amendments to aid P retention could also be incorporated at the time of tillage (e.g.,
464 Summers *et al*. 1996). However, the impact of mixing soil high in P lower into the profile
465 on the loss of P into shallow water tables may require consideration in some instances (e.g.
466 the dairy farm in our study). Interestingly, soil inversion may have desirable side-effects
467 such as those shown for 'clay delving' where bringing subsoil clay to the surface improves
468 profile wettability and reduces preferential flow (Betti *et al.* 2015). Indeed, this sort of
469 'strategic tillage' is increasingly considered as beneficial for minimum tillage cropping
470 systems (Dang *et al.* 2015), which also accummulate P in the top 5 mm of soil (Vu *et al*.
471 2009). However, the reduction in nutrient stratification from tillage or soil inversion will
472 dissipate over time (Nash *et al*. 2015). For instance, McLaughlin *et al*. (1990) rotary-hoed
473 the top 100 mm of soil under an annual pasture, applied 250 kg ha^{-1} $year^{-1}$ of
474 superphosphate, and then cut and removed plant shoots annually. After seven years,

475 stratification was similar to that in pastures that were not rotary hoed. Hence, in the Peel-
476 Harvey coastal catchment, the ideal frequency of tillage or soil inversion conducted to
477 reduce nutrient stratification would need to be determined.
478
479 *Limitations to pasture growth*
480 Whilst interpretation of our shoot nutrient concentration data must be undertaken with care
481 as the botanical composition of the pastures varied, they do suggest S and N limitations to
482 pasture growth at the beef farm in September and an N limitation at the dairy farm (Weir
483 and Cresswell 1994). This is consistent with the findings of Weaver and Reed (1998) who
484 sampled soils across the south coast region of Western Australia finding that two thirds of
485 high P status soils were deficient in S and a quarter deficient in K. Note that the
486 proliferation of kikuyu (*P. clandestinum* Chiov.) may explain the low shoot P
487 concentrations at the beef midslope site (Fulkerson *et al.* 1998). Limitations to pasture
488 growth from S, K and N, along with the over-application of P fertiliser, may have been the
489 cause of the high concentrations of P (>4 mg g^{-1}) in pasture shoots at the dairy and beef
490 upslope sites, and created a large pool of readily-leachable P in the living pasture shoots
491 (Bromfield and Jones 1972; McDowell *et al.* 2007). Addressing these nutrient limitations
492 may result in dilution of shoot P concentrations and potentially reduce the proportion of
493 shoot P consisting of reactive/soluble P; it would also improve pasture production.
494
495 **CONCLUSIONS**
496 We found pronounced stratification of P, K and S in the top 100 mm of the soil profile and
497 very high concentrations of P at the soil surface, particulary at the dairy. The litter on top
498 of the soil surface and pasture shoots also had high P concentrations and this was most
499 pronounced at the dairy. Differences among the three sampling times in concentrations of

P, K and S in soil were generally small and largest in the surface layers. Some large fluctuations in extractable soil P concentration at the dairy could not be readily explained. Thus, it seems that there are gaps in our understanding of P cycling in these pastures and, particularly, of the role of plants in P cycling. Nevertheless, together, the high concentrations of P at the soil surface and in the litter and pasture shoots undoubtedly constitute a source of risk for the movement of P off farmland. While there are many management practices that can be, or have already been, adopted to reduce P movement our results suggest three areas that merit further investigation: (1) use of pasture legumes that require less P for maximum yield and have lower shoot P concentrations than current cultivars; (2) soil tillage or inversion to reduce P stratification; and (3) capacity to reduce shoot P concentrations through reduced P-fertiliser application coupled with removal of K, S and N limitations to pasture growth.

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679 Table 1. Monthly rainfall totals in 2012 and the long-term average (LTA) monthly rainfall totals and monthly maximum and minimum
680 temperatures at Waroona, the closest town to the farms (Bureau of Meteorology, 2016). Months when sampling occurred are
681 highlighted. *=missing data

		J	F	M	A	M	J	J	A	S	O	N	D	Total
Rainfall (mm)	2012	2	5	0	45	94	214	47	117	156	37	75	*	794
	LTA	12	14	21	54	140	205	188	149	92	57	37	14	992
Maximum temperature (°C)	LTA	30	30	27	23	19	16	15	16	17	20	24	27	
Minimum temperature (°C)	LTA	14	15	13	10	8	7	6	6	6	8	10	13	

682

683 Table 2. Outcomes of three-way ANOVAs on extractable and total phosphorus (P), P-buffering index (PBI), sulfur (S), extractable potassium
684 (K), pH and organic carbon; the factors were depth (0–10, 10–20, 20–30, 30–40, 40–50, 70–80, 90–100 mm), sampling time (March, June,
685 September) and site (beef midslope, beef upslope, dairy farm) and their interactions.

	Depth (D)	Sampling time (T)	Site (S)	D x S	T x S	D x T	D x T x S
Log_{10}Extractable P	v.r.=34, P<0.001	n.s.	v.r. = 17, P<0.001	v.r.=2, P=0.043	v.r.=3, P=0.038	n.s.	n.s.
Log_{10}Total P	v.r.=30, P<0.001	v.r.=4, P=0.018	v.r. = 7, P=0.001	n.s.	n.s.	n.s.	n.s.
Log_{10}PBI[1]	n.s.	n.s.	v.r. = 6, P=0.004	n.s.	n.s.	n.s.	n.s.
Log_{10}S	v.r.=14, P<0.001	v.r.=5, P=0.01	v.r. = 75, P<0.001	n.s.	v.r.=3, P=0.015	n.s.	n.s.
Log_{10}Extractable K	v.r.=46, P<0.001	v.r.=11, P<0.001	v.r. = 30, P<0.001	n.s.	v.r.=3, P=0.021	n.s.	n.s.
Antilog pH	v.r.=17, P<0.001	v.r.=47, P<0.001	v.r. = 36, P<0.001	v.r.=3, P<0.001	v.r.=26, P<0.001	v.r.=3, P<0.001	v.r.=2, P=0.005
Log_{10}Organic carbon	v.r.=8, P<0.001	v.r.=4, P=0.013	v.r. = 20, P<0.001	n.s.	v.r.=3, P=0.03	n.s.	n.s.

686 n.s., not significant, v.r., variance ratio
687

688 Table 3. Dry mass and Colwell-extractable and total phosphorus (P) concentrations of litter on the soil surface
689 in March and September and concentrations of P, potassium (K), sulfur (S) and nitrogen (N) of pasture shoots
690 in September.

	Beef midslope		Beef upslope		Dairy farm		Statistical effects		
				Litter on soil surface					
	March	Sept	March	Sept	March	Sept	Site	Sampling time	Site × time
Dry mass (t ha^{-1})	2.28	0.17	1.58	0.17	1.45	1.25	n.s.	v.r.=38, *P*<0.001	v.r.=8.3, *P*=0.006
Extractable P (mg kg^{-1})	315	340	309	559	543	488	n.s.	n.s.	n.s.
Total P (mg kg^{-1})	525	534	775	708	838	826	v.r.=14, *P*<0.001	n.s.	n.s.
Extractable P (kg ha^{-1})	0.71	0.06	0.49	0.09	0.79	0.61	v.r.=4.4, *P*=0.039	v.r.=16, *P*=0.02	n.s.
Total P (kg ha^{-1})	1.20	0.09	1.22	0.12	1.22	1.03	n.s.	v.r.=31, *P*<0.001	v.r.=4.6, *P*=0.036
				Pasture shoots					
	June	Sept	June	Sept	June	Sept	Site	Sampling time	Site × time
P (mg g^{-1})	1.8	1.3	4.7	2.7	5.9	4.2	v.r.=40, *P*<0.001	v.r.=18, *P*=0.002	n.s.
K (mg g^{-1})	19.2	17.4	28.0	20.1	25.9	20.9	n.s.	n.s.	n.s.
S (mg g^{-1})	2.4	1.5	2.6	1.2	2.5	3.0	v.r.=6.8, *P*=0.016	v.r.=8.1, *P*=0.019	v.r.=7.4, *P*=0.013
N (mg g^{-1})	–	13.2	–	11.4	–	26.6	v.r.=60, *P*=0.004	–	–

691 Phosphorus: Deficient - <2.0; Low - 2.2-2.3; Normal - 2.5-0.5 mg g^{-1}. Potassium: Normal 11-25 5 mg g^{-1}. Sulfur: Deficient - <2.2;
692 Low - 2.2-2.3 mg g^{-1}; Normal - 2.5-0.4 mg g^{-1}; Nitrogen; no values given for deficient, Low 30-32, Normal 33-55 mg g^{-1} (Weir and
693 Cresswell (1994) for subterranean clover)

694 n.s., not significant, v.r., variance ratio

695 **Figure captions**

696 Figure 1. The location of the beef and dairy farms in the Peel Harvey catchment (A) and the
697 location of the two sites at the beef farm (B) and the single site at the dairy farm (C). Arrows
698 in (B) and (C) indicate surface run-off direction following topographic slope.

699

700 Figure 2. The concentration of extractable phosphorus (P) (A) and total P (B), and the P-
701 buffering index (PBI) (C) in 10 mm increments of soil profiles sampled to 100 mm depth for
702 three sites (mean ± s.e., n=3). Note that for C), the three individual replicate quadrats are
703 shown for the beef farm sites to illustrate the variability among replicates.

704

705 Figure 3. The concentration of sulfur (S) (A) and extractable potassium (K) (B) in 10 mm
706 increments of soil profiles sampled to 100 mm depth for three sites (mean ± s.e., n=3).

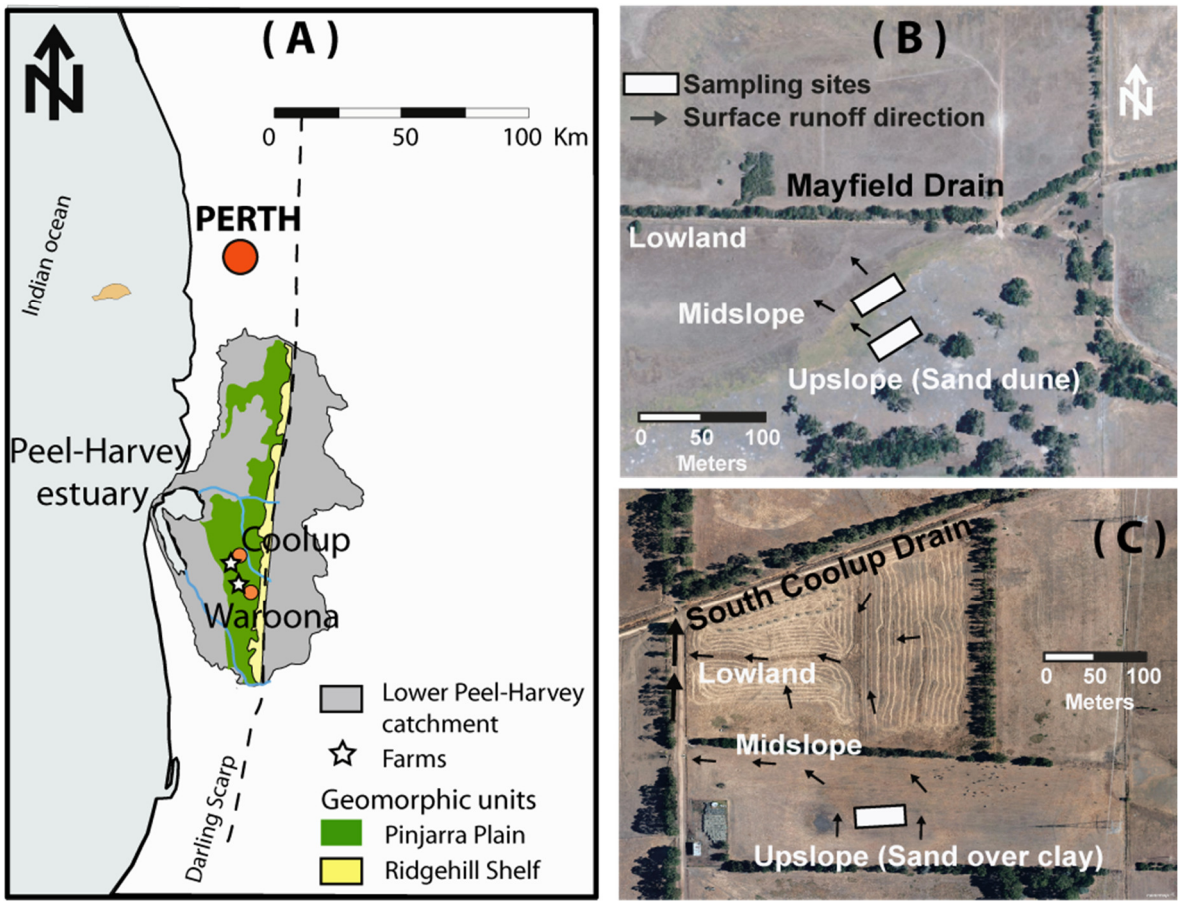
707

708 Figure 4. The pH in $CaCl_2$ (A) and organic carbon concentration (B) in 10 mm increments
709 of soil profiles sampled to 100 mm depth for three sites (mean ± s.e., n=3).

710

711 Fig 1

712



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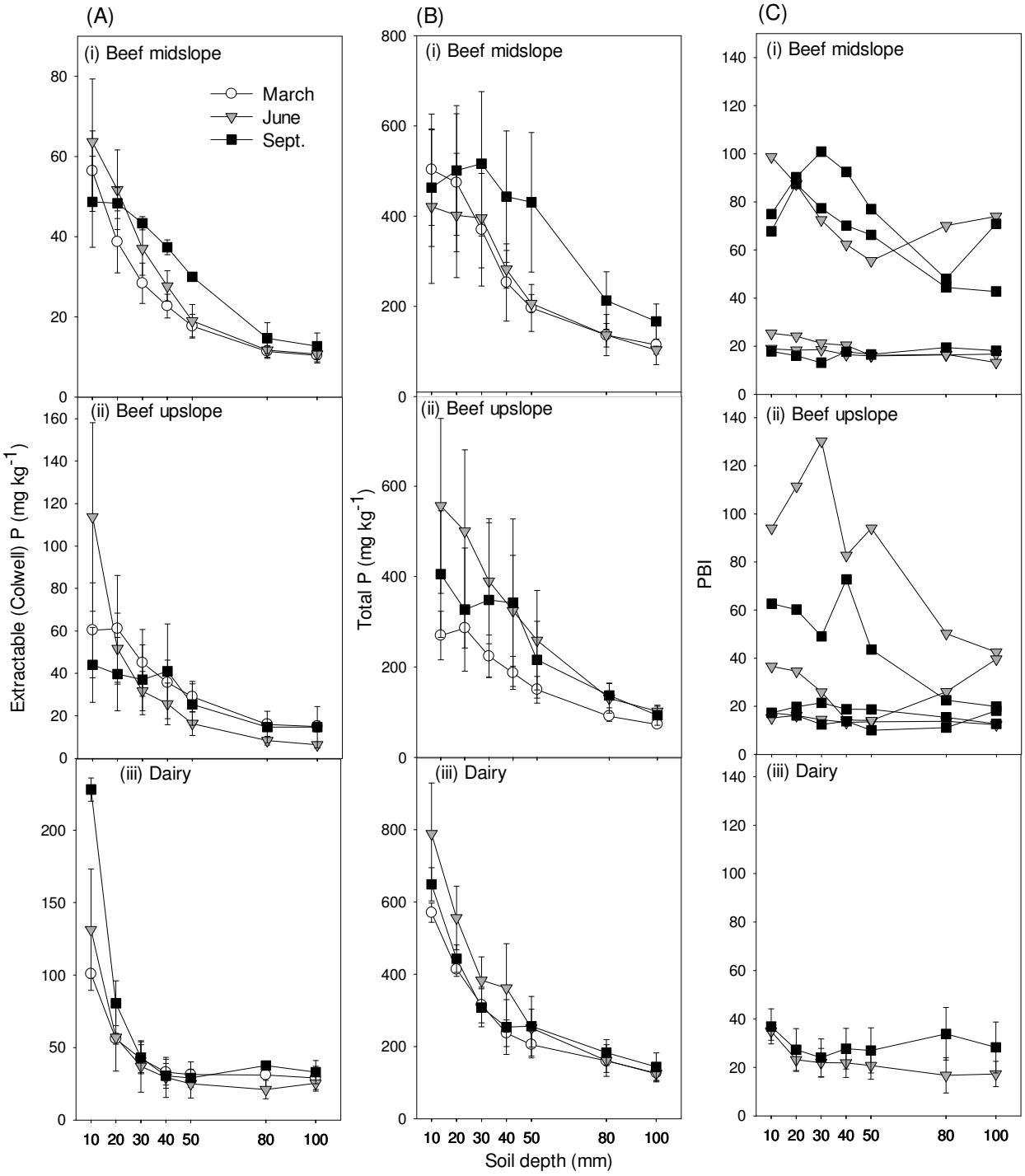
724 Fig 2 revised



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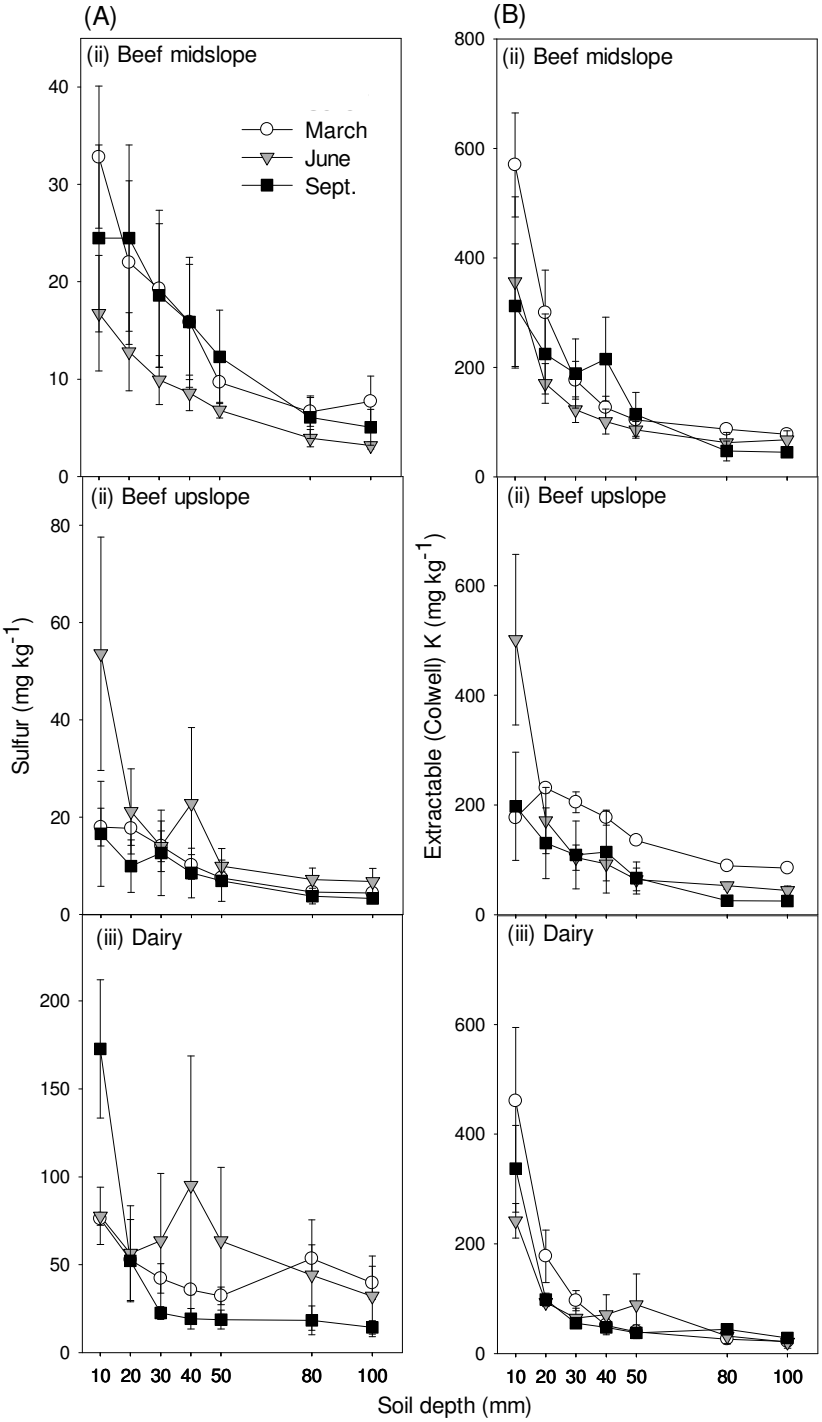

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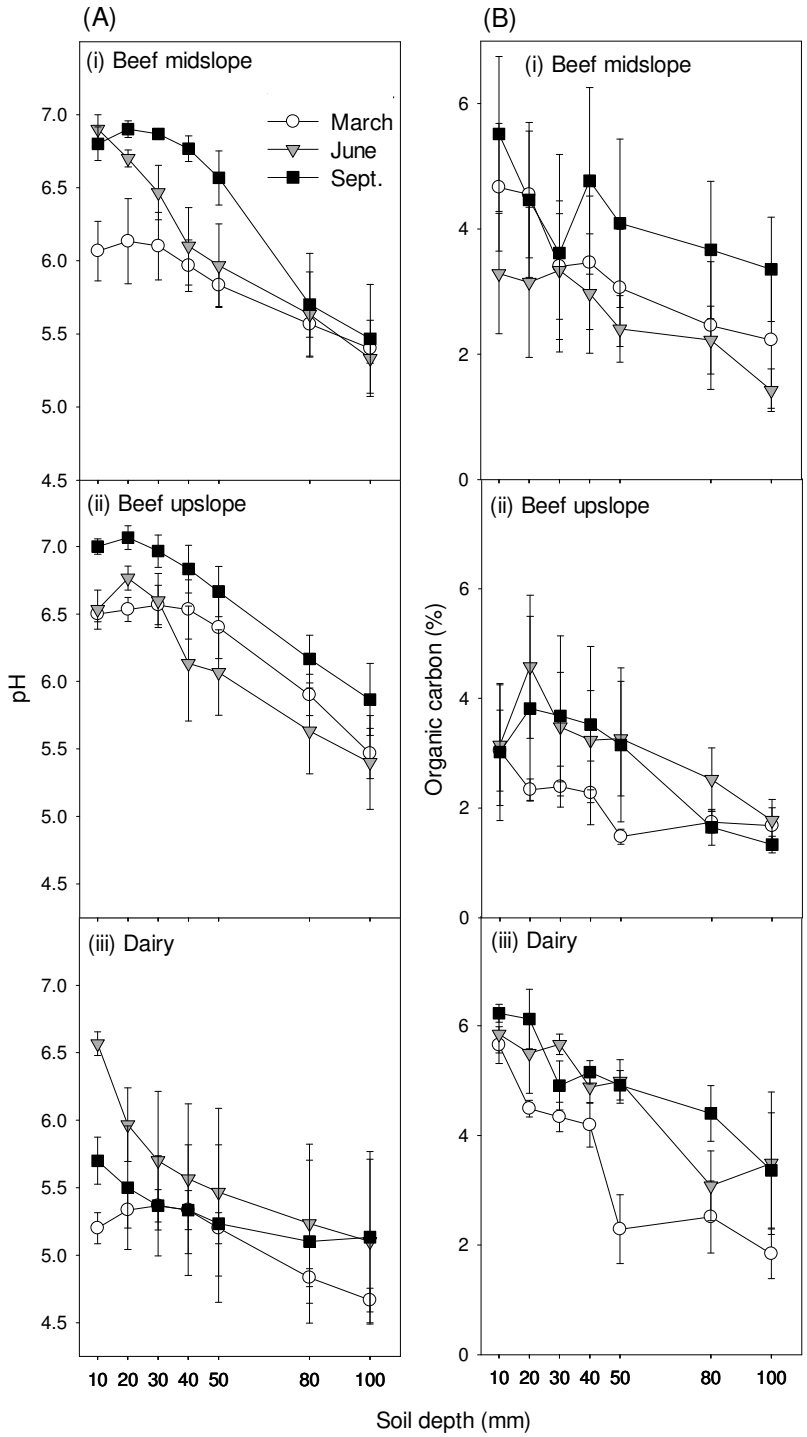
728 Fig 4 REVISED



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